LACK OF POSITIVE CORRELATION BETWEEN CELL RESPIRATION AND CYTOCHROME CONTENT IN GALACTOSE-GROWN *SACCHAROMYCES CEREVISIAE*

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1. Introduction

Facultatively anaerobic yeasts are ideal for studies on mitochondriogenesis. It is usually assumed that the level of cell respiration in these organisms is closely correlated with the content of mitochondrial redox enzymes. Indeed, numerous data [1–4] testify to an at least qualitative agreement between the changes in the mitochondrial respiratory activities and in the cytochrome content that occur during growth in culture or upon alterations of the environmental conditions (glucose repression–derepression, deaeration–respiratory adaptation). The results of the present study, however, demonstrate the lack of positive correlation between these two properties insofar as the whole cell is concerned. It is also shown that the changes in the cytochrome content of the mitochondrial fraction may not be indicative of those in the overall cell cytochrome content. The probable nature of the described phenomena is discussed.

2. Materials and methods

The *Saccharomyces cerevisiae* strain used and the growth conditions were the same as described previously [5–7], except that 2% galactose served as a carbon source in the present study. The homogenate was prepared by the method of Tzagoloff [8] in a medium containing 0.55 M mannitol, 100 mM Tris–HCl, pH 7.5, 2 mM $MgCl_2$, 1 mM EDTA. The fraction of 'heavy' mitochondria and the total membrane fraction were obtained by centrifugation of the homogenate at 8000 *g* for 30 min and at 105 000 *g* for 45 min, respectively.

The difference (reduced minus oxidized) spectra of the intact cells were recorded at 77°K [9] in a DW-2 UV/VIS Aminco spectrophotometer equipped with a low-temperature attachment. The cells were suspended in 0.55 M mannitol, 0.05 M potassium phosphate buffer, pH 7.5, so as to obtain a concentration of 10^9 cells per ml (200 mg wet weight per ml). The light path was 2 mm and the band-width 1 nm throughout. Oxidation–reduction was performed by addition of hydrogen peroxide (0.1%) and sodium dithionite [10]. The cytochrome content at room temperature was measured in the same spectrophotometer. Cell respiration, NADH oxidase and succinate oxidase activities were measured polarographically [11]. Protein was determined according to Lowry et al. [12].

3. Results and discussion

3.1. *Development of cell respiration*

As can be seen in fig.1A (curve 2), during aerobic growth of *S. cerevisiae* in the galactose-containing medium cell respiration almost reaches its maximal value in the early exponential phase (after approx. 8 hr of growth) and then only slightly increases through the exponential phase. The resulting (after 15 hr of growth) value of the cell respiration is equal to that of the inoculum. The development of cell respiration in the present experimental conditions thus follows a different pattern than during growth on low concentrations of glucose, where it follows the sigmoidal increase of the biomass [7].

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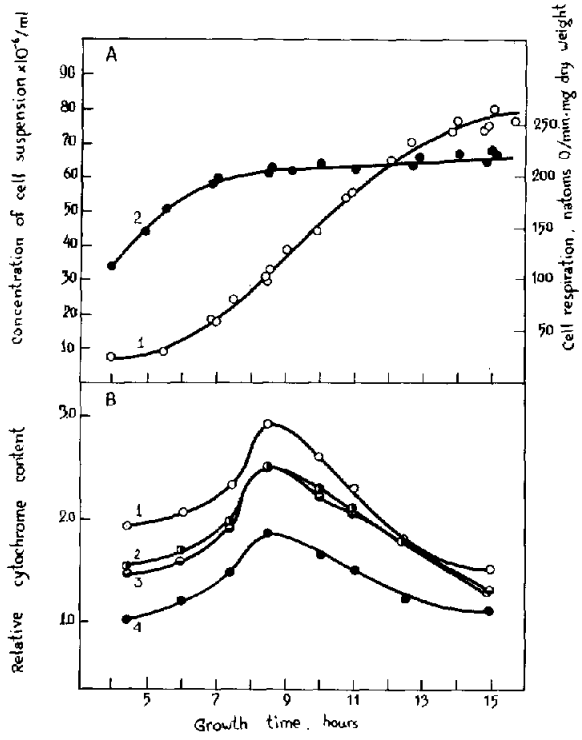

Fig.1. *S. cerevisiae* aerobic growth in galactose-containing medium and variations in the cell cytochrome content. (A) Growth in culture. Curve 1, concentration of cell suspension. 10^6 ml. Curve 2, cell respiration, natoms O/min. mg dry weight cells. (B) Relative cytochrome content of the intact cells. 1, cytochrome *c*, 2, cytochrome c_1, 3, cytochrome *b*, 4, cytochrome aa_3. Cytochrome content in the cells of the inoculum is assumed to be equal to 1.0. For experimental details see Methods.

3.2 *Changes in the cell cytochrome content*

In order to study the development of the cell respiratory apparatus of galactose-grown *S. cerevisiae*, the low temperature (77°K) difference spectra of the intact cells were recorded at various times during growth in culture. Figs. 1B and 2 demonstrate that the cell cytochrome content reaches its peak value by 8.5 hr of growth, up to that moment following the increase in the cell respiration. But this is where the correlation ends. Most strikingly, the cell cytochrome content decreases then through the exponential phase by a factor of 1.7–2 (fig. 1B); meanwhile the cell respiration remains at least constant or even slightly increases (fig. 1A). These results are in a sense in

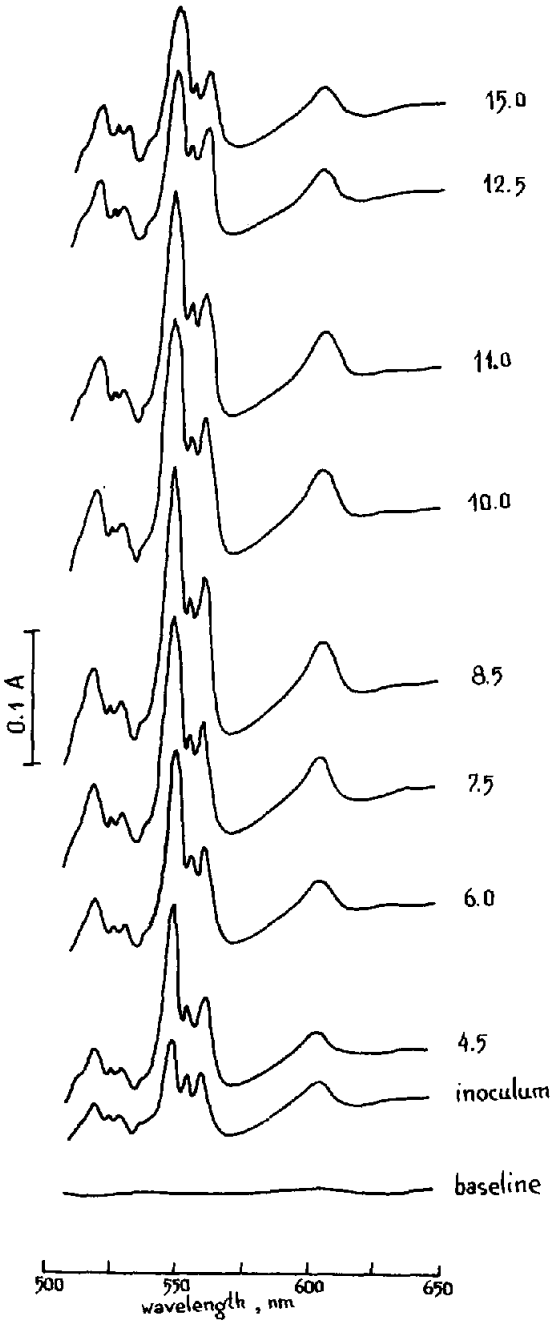

Fig.2. Low temperature (77°K) difference cytochrome spectra of the intact cells. Numbers on the right denote time of growth in culture, (hr). Measurement conditions are described in Methods.

line with the data of Cartledge et al. [13] on the variations in the cytochrome content of *S. carlsbergensis* in the course of respiratory adaptation. It is apparent from the latter that the homogenates of the cells after 6 hr of adaptation possess a higher cytochrome content than the homogenates of the aerobically grown culture. This phenomenon seems to have passed unobserved by the authors.

According to present day concepts, the respiratory apparatus of the facultatively anaerobic yeast undergoes a certain 'improvement' during the exponential phase of growth. It must be emphasized that in this particular case the improvement is apparently realized on the principle of intensification (i.e. increase of the ratio of the respiratory activity to the cytochrome content), and not expansion [2,14–16], of the cell respiratory machinery. Thus, the highest cytochrome content does not unambiguously testify to the most efficient respiratory apparatus.

The lack of positive correlation between cytochrome content and cell respiration is also shown by the fact that after 4–5 hr of growth the cells display only 60–70% of the maximal respiratory activity (i.e. that in the inoculum or after 15 hr of growth, see fig. 1A), though possessing a higher cytochrome content than the inoculum (Fig. 1B, 2).

3.3. *Cytochrome content of the total membrane fraction and 'heavy' mitochondria*

As was shown in the previous section, and 'excess' amount of cytochromes is formed during the early exponential phase of *S. cerevisiae* aerobic growth in galactose-containing medium. This 'excess' apparently disappears by the end of the exponential phase. It

Table 1
Cytochrome contents and respiratory activities of *S. cerevisiae*

	Total membrane fraction				Total homogenate	
	Overall cytochrome content (nmoles/g dry weight cells)		Specific cytochrome content (nmoles/mg fraction protein)		Homogenate respiratory activities (nmoles substrate/min. mg dry weight cells)	
	cyt. *b*	cyt. aa_3	cyt. *b*	cyt. aa_3	NADH	succinate
Early exponential phase (8.5 hr of growth)	69.2	56.6	0.298	0.243	35.4	19.3
Late exponential phase (15 hr of growth)	39.4	34.1	0.290	0.245	37.2	20.5
	Heavy mitochondrial fraction					
	Overall cytochrome content (nmoles/g dry weight cells)		Specific cytochrome content (nmoles/mg fraction protein)		Respiratory activities (nmoles substrate/min. mg protein)	
	cyt. *b*	cyt. aa_3	cyt. *b*	cyt. aa_3	NADH	succinate
Early exponential phase (8.5 hr of growth)	19.6	14.2	0.51	0.37	462	188
Late exponential phase (15 hr of growth)	24.9	22.4	0.65	0.56	616	228

Subcellular fractions. Isolation conditions for the fractions are described in Methods. The following extinction coefficients were used for cytochrome measurements: cytochrome *b* (562–575 nm), 19.1 $mM^{-1} \times cm^{-1}$; cytochrome aa_3 (605–630 nm), 12$mM^{-1} \times cm^{-1}$ [17].

seemed inportant to try and elucidate the nature of this phenomenon. With this in view, the total 150 000 *g* membrane fraction and the heavy mitochondrial (8000 *g*) fraction of the yeast cells were studied at growth times corresponding to the maximal and minimal cell cytochrome concentrations (8.5 and 15 hr, respectively).

It can be seen in table 1 that the homogenates of the cells harvested after 8.5 and 15 hr of growth display similar NADH oxidase and succinate oxidase activities (these activities, sensitive to cyanide (10^{-3} M) and antimycin A (0.1–0.2 μg/ml), represent the total respiratory activities of the mitochondrial structures contained in the cell-free preparation). This is entirely consistent with the relation of the levels of cell respiration at the respective times (fig. 1A). It is further shown that the overall cytochrome content of the 105 000 *g* membrane fraction isolated from the homogenates decreases by the end of the exponential phase by 40–45%, i.e. like that of the intact cells (figs. 1B, 2). Moreover, the specific cytochrome content of the total membrane fraction is the same at 8.5 and 15 hr (see table 1), and the fall in the overall amount of cytochromes is the result of the diminution of the quantity of the membrane protein per cell. The latter may obviously be attributed either to breakdown and elimination of a certain part of the mitochondrial membranes (as well as, possibly, other membranes), or to a decrease in the rate of synthesis and/or assembly of mitochondrial components, resulting thus in the 'scattering' of mitochondria (i.e. diminution of the amount of mitochondrial material per cell) during cell divisions, or both these processes. These alternatives cannot be chosen between at present. Still, some information on the nature of this phenomenon can be furnished by the data pertaining to the heavy mitochondrial fraction. The amount of the 8000 *g* fraction protein per cell is the same at the early and late exponential phases, while the specific cytochrome content of the heavy mitochondria increases by 15 hr of growth, as do their NADH oxidase and succinate activities (table 1). Hence the contribution of heavy mitochondria to the overall cytochrome content increases during the exponential phase from 25–30% to 65%. It is therefore obvious that the 'scattering' of mitochondria, if it occurs, has to be accompanied by a certain reorganization of the mitochondrial population. The possibility of simple 'fusion' of the 'light' membranes or their 'insertion' into heavy mitochondria can be almost certainly ruled out because these mechanisms are not consistent with the increase in the specific cytochrome content of the latter fraction. Thus it can be suggested that the 'light' membranes are somehow 'taken apart', possibly providing the material for the improvement of the heavy mitochondria. Of course, other explanations of the observed phenomena are not excluded, and further studies along this line seem to be quite promising.

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